

## **REMARKS**

The amendments are made without prejudice or disclaimer of the canceled subject matter. Applicants do not imply by the amendments any agreement with the present rejections. Applicants thus reserve the right to file a continuing or divisional application on any subject matter canceled by way of amendment. Applicants respectfully request reconsideration and reexamination of the present application in light of the foregoing amendments and following remarks.

### **1. Status of the Claims**

Following entry of the amendment, claims 1, 4-6, 10-12, 14, and 16 are pending and stand rejected. Claims 2, 3, 7, 9, 13, 15, and 17 are canceled without disclaimer or prejudice. No claims are withdrawn.

### **2. Support for the Amendments**

It is well established that a specification may support a claim amendment implicitly, in the absence of *ipsis verbis* support. *See, e.g., In re Wright*, 9 U.S.P.Q.2d 1649, 1651 (Fed. Cir. 1989). In the present case, implicit support for a kit comprising the recited components in separate containers is found in the claims as originally filed and the specification at page 17, line 13, *et seq.*, including page 17, lines 26-27, page 18, lines 4-6. *See also* Part 5, *infra*, regarding the interpretation of a “kit” as a container.

None of the present amendments imply acquiescence with any aspect of the rejection, including the Office’s interpretation of “destaining” or the Office’s interpretation of about “about 2 ng” as equivalent to 500 ng.

### **3. Withdrawal of Previous Rejections**

The Office notes that rejections not restated in the Office Action are withdrawn.

**4. Rejections of the Claims Under 35 U.S.C. § 112, First Paragraph**

Claims 1-17 stand rejected under 35 U.S.C. § 112, first paragraph, as allegedly directed to impermissible new matter and to subject matter allegedly inadequately described in the specification. The Office bases both rejections on the allegation that the specification does not adequately describe a genus of compounds that can stain “about 2 ng” of a protein. The phrase “wherein the staining reagent may be used to detect about 2 ng of a protein” does not appear in the present claims, mooting the rejections. Applicants’ amendment does not imply acquiescence to any aspect of the rejections.

**5. Rejection of the Claims Under 35 U.S.C. § 102(a)**

Claims 14-17 stand rejected under 35 U.S.C. § 102(a) as being allegedly anticipated by Bayramoğlu *et al.*, *Chem. Eng. Sci.* 57: 2323-34 (July 2002) (“Bayramoğlu”). The Office bases the rejection on a particular interpretation of the claim term “kit.”

Applicants traverse the rejection. During prosecution, the Office must apply the broadest reasonable meaning of the claim terms in their ordinary usage as they would be understood by one of ordinary skill in the art, taking into account definitions or other teachings in the specification. *See In re Morris*, 44 U.S.P.Q.2d 1023, 1027 (Fed. Cir. 1997) (setting forth the “broadest reasonable interpretation” standard). In the present case, the noun “kit” has two representative meanings in ordinary usage:

- (1) a set of articles or implements used for a specific purpose, or
- (2) a container for such a set.

*See* The American Heritage College Dictionary, 4<sup>th</sup> ed., Houghton Mifflin Co. (2004). The artisan in the relevant field typically interprets “kit” according to the second definition. For example, corporations commonly sell “kits” containing reagents. *See, e.g.*, Exhibits 1-3, attached hereto.

The Office, however, applies the first definition or its equivalent. The Office alleges that Bayramoğlu teaches a “kit,” because Bayramoğlu allegedly brings together or gathers all the components of the claim. Having thus interpreted “kit,” the Office discounts that fact that the

disclosed dye is conjugated to Bayramoğlu's membrane. The Office reasons that Bayramoğlu gathered the membrane and dye together—thus creating a “kit”—before the dye is conjugated to the membrane.

Without acquiescing to the propriety of the Office's claim interpretation, Applicants' amendment clarifies that the second definition applies to the claimed kits. That is, the “kit” in the claims is a “container” that comprises the recited components independently packaged within a single container. Applicant has stated the meaning that the claim terms are intended to have, so the Office must examine the claims with that meaning. *See In re Zletz*, 13 U.S.P.Q.2d 1320, 1322 (Fed. Cir. 1989).

Giving “kit” its intended and recited meaning, Bayramoğlu's disclosure does not anticipate the present claims. To establish a prima facie case of anticipation, a single prior art reference must teach each and every element of the claimed invention, either explicitly or inherently. *Verdegaal Bros. v. Union Oil Co. Cal.*, 814 F.2d 628, 631, 2 U.S.P.Q.2d 1051, 1053 (Fed. Cir. 1987). Bayramoğlu, however, does not teach a kit for visualizing a protein bound to a protein-binding membrane comprising, independently packaged within a single container: (1) one or more protein-binding membranes; and (2) a staining reagent comprising at least one compound of formula I, or a salt of such a compound. Because Bayramoğlu does not teach each and every element of the claims, Bayramoğlu does not anticipate the presently claimed kit. The rejection accordingly should now be withdrawn.

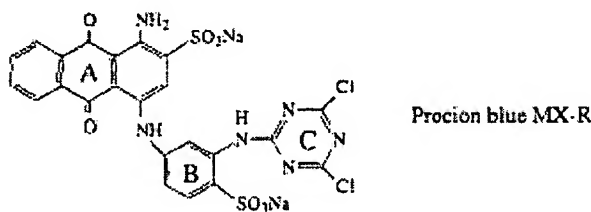
**6. Rejection of the Claims Under 35 U.S.C. § 103(a)**

Claims 1-17 are rejected under 35 U.S.C. § 103 as allegedly obvious over U.S. Patent No. 6,174,729 (“Alam”) in view of Hopwood *et al.*, *Histochem. J.* 5: 391-403 (1973) (“Hopwood”) and Miyagi, *Seibutsu Butsuri Kagaku* 19: 129-37 (1975) (Abstract) (“Miyagi”). Applicants traverse the rejection.

### The Cited Art

Alam teaches visualizing and quantifying a protein on a protein-binding membrane using Coomassie stain. Alam discloses detection of protein bound to a membrane by staining at col. 7, lines 44-58. Alam discloses suitable kits at col. 10, line 57, through col. 11, line 6. The Office acknowledges that Alam does not teach at least the use of a staining reagent comprising Reactive Brown 10.

Miyagi compares the ability of various dyes to stain membrane-bound proteins. The tested dyes are Coomassie Brilliant Blue G-250 ("title dye (I)"), Amido Black 10B, Ponceau 3R, Coomassie Brilliant Blue R-250 (II), and Procion Blue M-RS. Procion Blue M-RS is a dichlorotriazine dye. The cited art does not disclose the structure of "Procion Blue M-RS"; the precise structure of Procion Blue M-RS is unclear from the record. It stands to reason, though, that the structure of Procion Blue M-RS is different from Reactive Brown 10, given the differences in absorbed wavelengths of light between the structures (i.e., blue versus brown). This expectation is reinforced by the structural dissimilarity between Reactive Brown 10 and Procion Blue MX-R (which may or may not be the same compound as Procion Blue M-RS):



Alderton *et al.*, *Eur. J. Biochem.* 233: 880-85 (1995), at Figure 1 (referencing Hanggi *et al.*, *Anal. Biochem.* 149: 91-104 (1985)).

Hopwood teaches "fixative properties of a number of mono- and dichlorotriazine dyestuffs," where the dyestuffs are used to cross-link proteins in thin slices of rat liver and kidney. Hopwood, Abstract, page 393, ¶¶ 2-3. Hopwood states: "The aim of the present work was to investigate the potential of chlorotriazines as fixatives for tissues." Hopwood, page 400, ¶3. Hopwood teaches the use of the following chlorotriazines:

<i>Colour</i>	<i>Symbol</i>
Brilliant Blue	H-3R
Brilliant Blue	M-R
Brilliant Blue	M-3G
Brilliant Orange	M-2R
Brilliant Red	M-2B
Brilliant Red	H-3B
Brilliant Red	M-5B
Brilliant Yellow	H-5G
Brilliant Yellow	M-4G
Brilliant Yellow	M-4R
Brilliant Yellow	M-GR
Brilliant Yellow	M-6G
Grey	M-G
Green	M-2B
Olive Green	M-3G
Red Brown	M-4R
Scarlet	M-G
Red	M-G

Hopwood, page 392. Neither Procion Brown MX-5BR nor Reactive Brown 10 is listed among these Procion dyes.

### **The Rejection and Response**

The legal standard for determining obviousness in this art is set forth, for example, in *Takeda Chem. Indus. Ltd. v. Alphapharm Pty. Ltd.*, 83 U.S.P.Q.2d 1169, 1174 (Fed. Cir. 2007), *In re Dillon*, 919 F.2d 688, 16 U.S.P.Q.2d 1897 (Fed. Cir. 1990), and *In re Grabiak*, 769 F.2d 729, 731-32 (Fed. Cir. 1985). In summary, structural similarity between claimed and prior art subject matter may create a *prima facie* case of obviousness, ***where the prior art gives reason or motivation to make the claimed compositions.*** See *Dillon*, 16 U.S.P.Q.2d at 1901 (determining the obviousness of selecting tetraorthoesters, given prior art teaching tri-orthoesters). The Office discounts this legal precedent on the basis that “the ordinary artisan is not required to make molecular adjustments to Coomassie Blue in the method of Alam to arrive at the claimed invention.” The Office appears to allege that the Federal Circuit limits the reach of the cited decisions to the issue of the obviousness of new compounds, rather than the obviousness of selecting between known compounds. The holding in *Dillon*, for instance, is not so limited.

In the present case, the Office alleges that it would have been obvious to use Reactive Brown 10 in the method of Alam, because Alam teaches the suitability of any stain. The Office

further alleges that the artisan would have known from Miyagi that dichlorotriazine dyes are suitable for staining proteins bound to membranes. The Office finally alleges that "Hopwood teaches that Procyon dyes such as Reactive Brown 10 are suitable for staining and visualizing proteins in tissue." Office Action, page 5 (emphasis added). The Office alleges that the artisan would have combined the references, based on the allegedly known suitability of dichlorotriazine dyes, including Reactive Brown 10, as suitable alternatives to Coomassie Blue for protein staining. Office Action, page 5.

In short, the Office alleges that it would have been obvious to use any dichlorotriazine dye to stain proteins. The claims, however, are directed to Reactive Brown 10, not to any dichlorotriazine dye. The Office has not made a case that the all dichlorotriazine dyes are so structurally similar that the artisan would have selected Reactive Brown 10 from among these dyes to stain protein.

### **Unexpected Results**

Further, the Office argues that the artisan would select any dichlorotriazine dye on the notion that the properties of these dyes are similar to Coomassie Blue, taught by Alam, in regard to their ability to stain proteins. Applicants have demonstrated, however, that Reactive Brown 10 shows unexpectedly superior results, even compared to other dichlorotriazine dyes, for the following reasons.

Miyagi states that Procion Blue M-RS, Amido Black 10B, and Ponceau 3R have a detection limit of 500 ng and that Coomassie Brilliant Blue G-250 and Coomassie Brilliant Blue R-250 have a detection limit of 100 ng. The specification compares the detection limit of Reactive Brown 10 with Amido Black 10B, Ponceau S, and Coomassie Blue R-250. *See, e.g.,* Specification, Example 1. For PVDF and nitrocellulose membranes, the specification discloses a detection limit for Amido Black 10B, Ponceau S, and Coomassie Blue R-250 similar to that obtained by Miyagi using filter paper or cellulose acetate membranes: 50 ng, 100 ng, and 50 ng, respectively. *See* Specification, Tables 1 and 2. From this evidence, it is apparent that Miyagi's method of detection is comparable, although apparently 5-10 times less sensitive, to that used in

the present specification. By contrast, Reactive Brown has a detection limit of 2 ng. See Specification, Tables 1 and 2. These data are summarized below:

Compound tested	Detection limit in Miyagi (ng)	Detection limit in Tables 1 and 2 (ng)
Amido Black 10B	500	50
Ponceau S or 3R	500	100
Coomassie Blue	100	50
Procion Blue M-RS	500	N.D.
Reactive Brown 10	N.D.	2

From the evidence on the record, the detection limit using Reactive Brown 10 is 250-fold lower than achieved with Procion Blue M-RS. Even compensating for the increased sensitivity in the assay disclosed in the specification, the detection limit using Reactive Brown 10 is at least 25-fold lower than achieved with Procion Blue M-RS. Notably, Reactive Brown 10 is significantly more sensitive than Coomassie Blue.

The evidence on the record does not suggest that the detection limit using Reactive Brown would be so low, or particularly less than Coomassie Blue. In fact, if the Examiner's allegations were true, the artisan would expect the various Procion dyes to display the same properties, in view of their common monochloro- or dichloro-triazine ring moieties. From Miyagi, the artisan would expect Procion dyes to be about **5-fold less sensitive** than Coomassie Blue, based on Miyagi's comparison of the detection limit using Procion Blue M-RS (500 ng) and Coomassie Blue (100 ng). Instead, the specification demonstrates that Reactive Brown 10 is **25-fold more sensitive** than Coomassie Blue (detection limit of 2 ng versus 50 ng). Neither Alam nor Hopwood discuss the relative sensitivity of Procion dyes. Accordingly, the increased sensitivity of Reactive Brown 10 relative to Coomassie Blue is unexpected.

Given the unexpectedly superior results obtained using Reactive Brown 10, the presently claimed method, compound, and kit are patentable over the combination of Alam, Miyagi, and Hopwood, even if a *prima facie* case of obvious were made. The rejection accordingly should be withdrawn.

**CONCLUSION**

Should the Examiner have any questions or comments regarding Applicants' response, he is asked to contact Applicants' undersigned representative. Please direct all correspondence to the below-listed address.

In the event that the Office believes that there are fees outstanding in the above-referenced matter and for purposes of maintaining pendency of the application, the Office is authorized to charge the outstanding fees to Deposit Account No. 50-0573. The Office is likewise authorized to credit any overpayment to the same Deposit Account Number.

Respectfully submitted,

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Appl. No. 10/525,168

Reply to Non-Final Office Action mailed September 30, 2008

## **Exhibit 1**



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## **Exhibit 2**

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<input type="checkbox"/>	<b>QIAGEN PCR Cloning Kit (40)</b>	For 40 reactions: 2x Ligation Master Mix (200 ul), pDrive Cloning Vector (2.0 ug), distilled water (1.7 ml)	231124	\$274.00	N/A*

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<input type="checkbox"/>	<b>MinElute Gel Extraction Kit (50)</b>	For gel extraction of up to 5 µg DNA fragments (70 bp to 4 kb) in low elution volumes. Contents: 50 MinElute Spin Columns, Buffers, Collection Tubes (2 ml)	28604	\$100.00	N/A*
<input type="checkbox"/>	<b>CompactPrep Plasmid Midi Kit (25)</b>	For fast purification of up to 10 mg Molecular Biology Grade. Contents: 25 CompactPrep Midi Columns, Extender tubes, Reagents, Buffers, 25 QIAfilter Midi Cartridges	12743	\$203.00	N/A*

<input type="checkbox"/>		batch purification of DNA fragments (40 bp to 50 kb) from agarose gels and from solutions. Contents: 1.5 ml suspension			
<input type="checkbox"/>	<a href="#">QIAprep Spin Miniprep Kit (250)</a>	For purification of up to 20 ug molecular biology grade plasmid DNA. Contents: For 250 high-purity plasmid minipreps: 250 QIAprep Spin Columns, Reagents, Buffers, Collection Tubes (2 ml)	27106	\$328.00	N/A*
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## **Exhibit 3**



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**Fig. 1:** Typical results with Sequenase



**Fig. 2:** Comparison of <sup>35</sup>S- and <sup>33</sup>P-labeled dATP.



**Fig. 3:** Long reads with Sequence Extending Mix.

### Tech Tips

Relief from Compression Artifacts in DNA Sequencing Using Formamide Gels  
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Use of Terminal Deoxynucleotidyl Transferase (TdT) to resolve BAFL's  
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The Sequenase™ Version 2.0 DNA Sequencing Kit features Sequenase Version 2.0 DNA Polymerase, the standard for high quality manual DNA sequencing<sup>(1)</sup>. Sequenase Version 2.0 DNA polymerase is a genetically engineered form of T7 DNA polymerase which retains extraordinary polymerase activity with virtually no 3'→5' exonuclease activity. It is highly processive, able to effectively incorporate nucleotide analogs for sequencing (dideoxy NTPs, α-thio dATP, dITP, 7-deaza-dGTP, etc.) and is not easily impeded by template secondary structure. The kit includes all the reagents necessary to achieve high quality results (Fig. 1). Use of the specially formulated buffers and mixes included in the kit will maximize yield of sequence information.

### Flexible Labeling

The kit can be used for either internal labeling with α-labeled dNTPs (Fig. 2) or with 5' end-labeled primers.

### Resolve Gel Compressions with dITP Nucleotide Mixes

The substitution of dITP (deoxyinosine triphosphate) for dGTP in the reaction mix eliminates the secondary structures that produce gel compressions. dITP forms fewer H-bonds with dCTP than does dGTP, so product is more readily denatured during gel electrophoresis. Hence, sequence data is free from gel-based compression artifacts and results are more accurate.

### Emphasize Sequence Close to Primers with Mn Buffer

Manganese (Mn) is added to emphasize sequence close to the primer, which may be weak if

insufficient DNA template is used.  $Mn^{++}$  increases the incorporation rate of dideoxynucleotides relative to deoxynucleotides. Thus, termination occurs earlier and more sequence is visible close to the primer<sup>(2,3)</sup>.

#### **Increase Read Length with Sequence Extending Mixes**

These mixes enable chain terminations to be extended to more than 3,000 bases from the primer. The use of these mixes provides a simple method to further extend the range of sequence, if needed (Fig. 3). Keep in mind that this degree of extension can reach well beyond the limits of any electrophoresis gel resolution, yet use of the mixes when combined with short and long gel runs can increase overall sequence yield.

#### **Eliminate Weak Bands with Pyrophosphatase**

Occasionally, weak bands may occur with prolonged reaction times (greater than 5 min)<sup>(4,5)</sup>, or when dITP is used in the sequencing reaction<sup>(6)</sup>. The addition of pyrophosphatase can prevent weak band intensities brought on by sequence-specific pyrophosphorolysis catalyzed by the polymerase.

#### **Convenient Enzyme Storage with Glycerol Enzyme Dilution Buffer**

Sequenase DNA polymerase can be pre-diluted to a working concentration for storage of the enzyme in this form. This eliminates the necessity of diluting the polymerase prior to each sequencing reaction. Also, the addition of glycerol enhances the stability of the enzyme in sequencing reactions. Note: Pre-dilution of the polymerase with this buffer results in higher glycerol concentration in the sequencing reaction. A Glycerol Tolerant Gel (GTG) Buffer (PN 71949 or PN 75827) must be used in the sequencing gel and buffer chambers to eliminate glycerol-induced distortion of bands at approximately 350 to 600 bases beyond the primer<sup>(7)</sup>. If this region is beyond your region of interest, Tris-Borate-EDTA (TBE) Buffer may be used.

#### **Kit Components:**

Sequenase Version 2.0 DNA Polymerase

Inorganic Pyrophosphatase

Enzyme Dilution Buffer

Glycerol Enzyme Dilution Buffer

Sequenase Reaction Buffer (5X)

Dithiothreitol Solution

Mn Buffer

Control DNA M13 mp18

Primer (-40 Universal)

Labeling Mix (dGTP, 5X)

ddGTP Termination Mix (for dGTP)

ddATP Termination Mix (for dGTP)

ddTTP Termination Mix (for dGTP)

ddCTP Termination Mix (for dGTP)

Sequencing Extending Mix (for dGTP)



Labeling Mix (dITP, 5X)  
ddGTP Termination Mix (for dITP)  
ddATP Termination Mix (for dITP)  
ddTTP Termination Mix (for dITP)  
ddCTP Termination Mix (for dITP)  
Sequence Extending Mix (for dITP)

Stop Solution  
Protocol Book

This kit and all the enclosed reagents should be stored frozen at -20°C (NOT in a frost-free freezer). Keep all reagents on ice when removed from storage for use. Sequenase Version 2.0 enzyme must be stored at -20°C. **Never store Sequenase enzyme in a frost-free freezer since the temperature rises above 0°C daily.** If enzyme dilution buffer (no glycerol) is to be used, only dilute the amount of enzyme which is to be used that day. Dilute into ice-cold buffer and keep on ice until use.

#### References:

1. TABOR, S. AND RICHARDSON C. C. (1989) *J. Biol. Chem.* **264**, 6447-6458.
2. FULLER, C. W. (1989) *Comments* **16**, No. 3, United States Biochemical Corp., Cleveland, OH.
3. TABOR, S. AND RICHARDSON, C. C. (1989) *Proc. Nat. Acad. Sci. USA* **86**, 4076-4080.
4. RUAN, C. C., SAMOLS, S. B. AND FULLER, C. W. (1990) *Comments* **17**, No. 1, United States Biochemical Corp., Cleveland, OH.
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